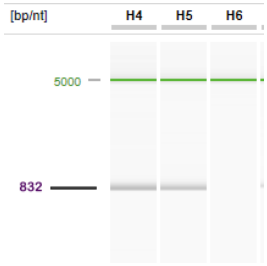
Nov 26, 2019

Aichivirus 3C3D RT-PCR



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We use this protocol and it's working

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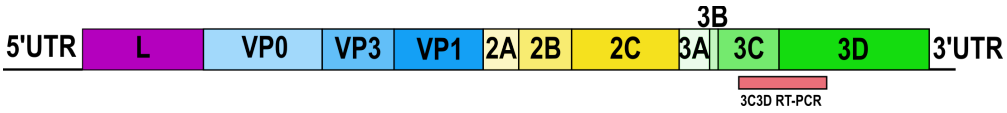
Abstract

This RT-PCR will detected Aichivirus A from human samples. It spans the junction region of 3C and 3D and is used for genotyping.



Schematic of Aichivirus A with the 3C3D RT-PCR target region.

Guidelines

Method assumes the user is familiar with the thermocycler and software used to run the protocol.
Mix preparation should occur in a different laboratory or room to the amplification or post-PCR area.

Materials

STEP MATERIALS

- 5ml Ethidium Bromide Solution [0.625mg/ml] **G-Biosciences Catalog #**R041
- Agarose low EEO (Agarose Standard) **AppliChem Catalog #**A21140100
- 100bp DNA Ladder, 250ul (50 lanes) **Promega Catalog #**G2101
- SuperScript™ III One-Step RT-PCR System with Platinum™ Taq High Fidelity DNA Polymerase **Thermo Fisher Scientific Catalog #**12574035

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Troubleshooting

Oligonucleotide sequences

1

	Name	5'-3'
	AiV-6213F	ACTGGGCCACCCTCCAGACG
	AiV-7044R	GGTTGATTTCAGCTTGGAGTTC

Reaction set-up

2

- Prepare sufficient for number of reaction plus a 'dead volume', usually 2 extra. Adjust as necessary if using a robotic dispenser.

SuperScript™ III One-Step RT-PCR System with Platinum™ Taq High Fidelity DNA Polymerase **Thermo Fisher Scientific Catalog #**12574035

	Reagent	Vol (µL) x1	Final reaction concentration
	Nuclease free water	3.6	
	Primer AiV-6213F (20pmol/µl)	0.5	500nM
	Primer AiV-7044R (20pmol/µl)	0.5	500nM
	2 X Reaction mix	10	1X
	Superscript III RT/Platinum Taq HiFi enzyme mix	0.4	
	TOTAL VOLUME	**15**	

Dispense 15µL to each reaction well.

Add 5µL of template, extracted RNA, controls or NTC (nuclease-free water).
Total reaction volume is 20µL

Amplification

3 The assay has been used with Eppendorf thermocyclers.

PCR cycling times

	1 cycle	40 cycles	1 cycle	Hold
	50° C 30 minutes	94° C 15 seconds	68° C 5 minutes	15°C
	94° C 2 minutes	50° C 30 seconds		
		68° C 60 seconds		

4

Amplified products are analysed by gel electrophoresis or equipment such as a QIAxcel.

For gel electrophoresis, a 1.5% agarose gel with ethidium bromide was made using the following recipe.

	Reagent	**Volume**
	Agarose	1.5g
	0.5 X TBE buffer	100 ml

Boil in microwave for 1.5 -2 minutes until agarose powder is dissolved.
Add 2 drops of ethidium bromide (0.625mg/ml) and mix before pouring warm into the gel form with a comb.
Leave for approx 30minutes at room temperature to set.

Note

For the novice user to gel electrophoresis there a detailed protocol at https://dx.doi.org/10.17504/protocols.io.s38egrw that will give you further guidance.

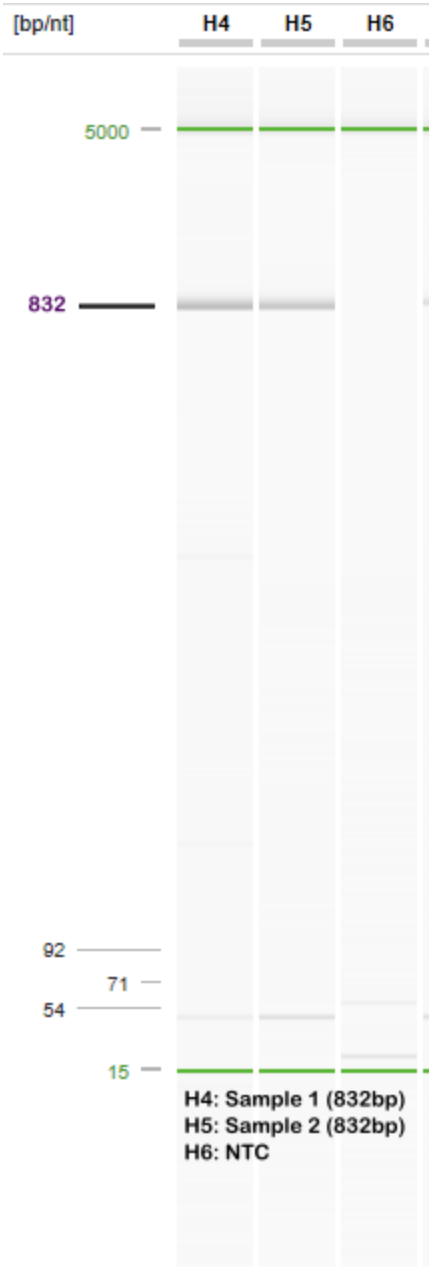
Experienced users may run their favorite gel recipe with appropriate dye. The aim is to visualise the amplified products. Gel running times and voltage can be adjusted depending on the equipment used.

5ml Ethidium Bromide Solution [0.625mg/ml] **G-Biosciences Catalog #**R041

Agarose low EEO (Agarose Standard) **AppliChem Catalog #**A21140100

100bp DNA Ladder, 250ul (50 lanes) **Promega Catalog #**G2101

Place gel (on a tray) into the tank and cover with 0.5X TBE with ethidium bromide.
Mix 2µl of loading dye with10µl of amplified product and add to a well in the gel.
Mix 2µl of loading dye with 5µl of 100bp marker and add to a well in the gel. Ideally, the first and last lane but this is not essential.
Run the gel for 60-90 minutes at 80 volts or until the bands on the marker have separated adequately without run off the end of the gel.



Example of 3C-3D products analysed using a QIAxcel.

Expected result

Amplified products will produce a band of 832bp in size. No temple controls (NTC) should be not detected.